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BIOLOGICAL BULLETIN

CERTAIN HABITS, PARTICULARLY LIGHT REACTIONS, OF A LITTORAL ARANEAD.

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This tiny beach-comber has been identified by Mr. Nathan Banks as *Grammonota inornata* (Emert.), a species of theridiid, for which I am much indebted to him. It lives at Woods Hole, Mass., and in its vicinity, upon the sandy beaches along Vineyard Sound and Buzzards Bay. Here it is found, however, in only small scattered colonies for it occurs only on those sand beaches where the eel grass, thrown up by the sea, lies in more or less permanent lines upon the beach. Thus it does not exist upon gravelly or rocky beaches, nor yet upon sandy beaches devoid of the eel grass. The area of its distribution is limited to this cast-up eel grass, from a line slightly above high tide level landward as far as the patches of eel grass extend, a distance rarely exceeding ten or twelve feet and determined more or less by the degree of slope of the beach. In this peculiar home it obtains perpetual moisture and darkness, and does not migrate further up the beach nor into the salt marshes. In the same places occur a variety of animal forms in great individual abundance. Of this fauna the gammarids constitute the only truly marine element, the others being mainly terrestrial forms: certain other spiders, of which a drassid and the young of *Trochosa cinerea* are most abundant, staphylinid and chrysomelid beetles, an acarine, a pseudoscorpion and a chilopod. These are all normal constituents of this fauna, not waifs drifted in by the sea.[1]

My interest was drawn to them by the observation that when

[1] The elements of the "upper beach fauna" have been interestingly characterized by Davenport, "The Animal Ecology of the Cold Spring Sand Spit," Dec. Publ. Univ. Chicago, 1903. But he does not mention the particular species we are now considering.

they are disturbed by the removal of the eel grass, they all run directly landward provided only the sun is shining from an angle. Fifty or a hundred or more of these spiders may be set into commotion by the lifting up of a bunch of the eel grass, and under the conditions stated the large majority, sometimes all, of them invariably run in a straight course right up the beach and do not stop until they reach shelter beneath another mound of the eel grass. This habit is the more striking because none of the other species that live with them exhibit any such regular landward migration.

Direction and velocity of the wind have nothing to do with determining this course of running, for the landward migration occurs whether the wind blows upon the spiders from in front, or behind or from the side, or when there is no wind. Further, the spiders are not guided by any moisture sense, for (1) they do not regularly run landward when the sun is obscured, and (2) if the observer pours out sea water at a higher level and lets if flow down towards the spiders, they nevertheless continue their landward course. Again, this is not a geotropism, a tendency of the spiders to run up hill, nor an orientation to the rolling of sand grains. For the observer can dig a deep trench to landward of the spiders, and they will go down its declivity and up its opposite side without changing their course; or one can implant a board vertically in the sand in front of them, when they will climb up one surface of it and down the other without changing their general direction of movement.

Therefore the landward course of these spiders is not caused by any influence of wind or moisture or slope of the beach, but is probably a light reaction as the following observations would show.

When the sun is shining and when its rays come from an angle, at any time but noon, the spiders regularly run up the beach; this I have tested many times and on different beaches facing different points of the compass. That is, the spiders run up the beach whether the sun shines in front of, behind or to one side of them. But at noon, when the sun is nearly vertical, they run in all directions; and that they do also when the observer on a sunny day shades them with an umbrella.

Then a number of experiments were made to test the reactions of the spiders to the rays of an ordinary student oil lamp placed one foot or a foot and a half away from them, all these experiments being made at night. Numbers were caught and placed in small vials, which can be done without injury to them for they are hard-bodied. Then a piece of smooth white paper was laid horizontally upon the table, and kept free from sand or other particles; on this paper four quadrants were marked, the one nearest the lamp marked *A*, the one most distant from it *D* and the other two quadrants *C* and *B*. A vial was then uncorked and the spiders allowed to drop in succession gently upon the center of the paper, each falling slowly on its own drag line. With a pencil the course of each spider was then marked upon the paper, which made a permanent record of all movements of all individuals. On different nights a total of 107 spiders were thus tested, with the following results: 3 ran into quadrant *A*, directly towards the light; 45 ran into quadrant *D*, directly away from the light; 28 turned into quadrant *B* and 31 into quadrant *C*, these accordingly at right angles to the direction of the light. This proves negative phototropism to the rays of an oil lamp, for only 2.8 per cent. of the individuals ran towards the light. In these experiments it was purely a matter of chance how the spider was oriented when it first touched the paper. Of those that happened to touch it facing the light almost all moved through an arc of a circle so as to get into another direction and then continued their courses in almost straight lines; there was a marked turning away from the source of the light.

Another experiment was made with diffuse daylight. Eleven spiders were dropped in similar manner upon a piece of white paper placed on a table in the northwest corner of a room, sunlight entering the east window but not reaching the table. Ten of the spiders turned directly away from the sunlight, and one at right angles to it. This was then negative phototropism.

In a third experiment spiders were dropped upon a piece of white paper on which the sun shone directly, the sun being then near the meridian (10.45 A.M.). In this case ten moved towards the sunlight, one at right angles to it, and eight away from it.

This result seems at first sight to be contradictory to the preceding experiments. But I believe it is explainable by the nearly vertical position of the sun's rays; for on the beach, at noon when the sun is nearly vertical, the spiders do not run in any regular direction.

It thus seems that these spiders are decidedly negatively phototropic to lamplight and to diffuse daylight, when these impinge upon them from an angle; but that when sunlight falls upon them nearly vertically they do not orient themselves to it. How is it then on the beach that the spiders run so regularly landward whenever the sun is shining from an angle, irrespective of the position of the sun, which may be in front or behind or to the right or the left of them? One would not suppose it to be a case of negative phototropism when they run straight towards the sun. Yet I believe it is this nevertheless, in that the spiders may orient themselves not to the sun directly but to the light from the water. For the area of light in the sky and clouds above the water is certainly greater, and the light more intense, than that to landward, owing to the great amount of reflection from the water. It is well known that a man becomes more sunburnt upon the water, a test that the sunlight is more effective there. It would seem that only in this way can we correlate the experiments made with the oil lamp and with diffuse sunlight, with the observations recorded upon the sea beach. That animals react to area as well as to intensity of light is now well known. Thus G. H. Parker[1] showed that *Vanessa*, when in the open, always comes to rest with its head away from the sun, and that it thereby "reacts positively to large patches of bright sunlight rather than to small ones, even though the latter, as in the case of the sun, may be much more intense." Cole[2] has confirmed Parker's results, and concluded from his own painstaking observations on several forms, that animals with direction eyes, as those without eyes, respond to light intensity only; and that animals with image-forming eyes when positively

[1] "The Phototropism of the Mourning Cloak Butterfly, *Vanessa antiopa* Linn.," Mark. Anniv. Vol., 1903.

[2] "An Experimental Study of the Image-forming Powers of Various Types of Eyes," *Proc. Amer. Acad. Arts and Sci.*, 42, 1907.

phototropic react to the size of the luminous field or to definite objects in the visual field.

Our *Grammonota* probably possesses an image-forming eye, but it is negatively phototropic. Whether it reacts to intensity of the light or to its area remains to be determined. I have made no attempt to decide whether it reacts rather to the light rays than the heat rays, and I was not interested to determine this, for in the state of nature heat and light, so far as they affect these animals, are probably always associated.

Under natural conditions the masses of eel grass that shelter these spiders would be disturbed only by unusually high tides, perhaps occasionally also by violent winds. If the sun were shining during such a disturbance the spiders, from their negative phototropism, would attempt to run landward, and thus some of them might escape the action of the waves; but it is questionable whether this light reaction would be of any great benefit to them at times when the waves play havoc on the beach.

From the scattered distribution of these spiders one would suppose they might be readily transported by water. I accordingly made a few experiments to ascertain how sea water affects them. A number were dropped upon clean sea water placed in a glass dish; they were able to stand upon the surface film, but not to run unless there were fine dust particles upon it. But so soon as the water was violently agitated they sank below the surface and were unable to rise again. Therefore they could not long remain upon the top of a wave, but would become quickly submerged unless they clung to some floating vegetation. This is because the body is only slightly pilose. They become quiet after a few minutes of submergence, as though partially suffocated, yet they can withstand submergence for some hours. Thus I kept seven beneath water for five and a half hours, then placed them upon blotting paper to dry, when four revived; and I kept another lot beneath water for sixteen hours, and one of these revived after drying.

A number were placed in isolated vials, each with a few drops of sea water to furnish the necessary moisture, in order to observe the cocooning, but I was not so fortunate as to see this process. The cocoon is lenticular, snow-white and relatively

very large—its diameter considerably greater than the length of the spider. Nine cocoons were made in these vials, by as many females, and all at some time between 10:30 P.M. and 7 A.M., the time of cocooning would then seem to be nearly morning. On previous occasions I have indicated how regular this time is in spiders, and how it varies with different species.

Males are abundant through the summer months, but the mating was seen only in part although many of both sexes were kept in observation cages. A male and female were seen to move directly towards each other, and on meeting each elevated its head region so that the line of the body made an angle of about 45° with the floor, when the male extended his cephalothorax somewhat beneath and to one side of that of the female. This attitude is similar to that of *Dictyna*.

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PRELIMINARY NOTE ON THE ECOLOGY OF THE EARLY JUVENILE LIFE OF THE UNIONIDÆ.[1]

F. B. ISELY.

During the past four years the writer has given considerable time to the field study of the Unionidæ. A number of puzzling questions have arisen as to the relative importance of the various ecological factors that contribute to the environmental complex of these animals. Among the most important of these factors, we may mention bottom conditions, water content, stream volume, stream fluctuations, relation to fish and natural enemies.

In connection with this work, much difficulty was experienced in finding young mussels for study and experimentation. I have collected many specimens from the size of a nickel to a quarter, but mussels under the size of a dime have been rare. A number of experienced field workers have spoken to me of a similar difficulty in finding juvenile specimens.

In order to avoid any misunderstanding, I may state that by "early juvenile life" I mean the period following the time when the mussel completes the parasitic stage and leaves the fish to lead an independent life, until it is about the size of a dime or about fifteen millimeters in length. This would cover, in most species, approximately the first year of independent existence. Other periods may be designated as later juvenile and adult life.

The glochidium stage has received considerable study. Our knowledge of the parasitic period has been recently cleared up and extended by the careful investigations of LeFevre and Curtis.[2] The later juvenile and adult life has received the attention of hundreds of students. The scattering and meager references in the literature, to the early juvenile stages, give us little information on this important period in the life history of the mussel.

During the past summer, while working on the Red River Mussel

[1] Published by permission of the U. S. Commissioner of Fish and Fisheries.

[2] "Reproduction and Parasitism in the Unionidæ," George LeFevre and Winterton C. Curtis, *Journal of Experimental Zoölogy*, Vol. IX., No. 1, September, 1910, pp. 79–115.

Survey for the Bureau of Fisheries, I found a fairly good number of species in the early juvenile period of development. The distribution and ecology of these young mussels seem to me to be of special interest. Furthermore, the facts in regard to their habitat seem to clear up some ecological perplexities concerning the adult ecology of the Unionidæ.

Thirty-two specimens are here especially considered, these were attached to rocks and pebbles by a functional byssus. These mussels, representing nine species, were found in three different rivers and in four localities. The situations, however, were similar. Twenty-nine of these specimens weigh less than five decigrams; of this number twelve weigh under one decigram, and ten are under nine millimeters in length.

The thirty-two specimens represent the following species:[1] (1) *Lampsilis luteola*, two; (2) *Lampsilis fallaciosa*, one; (3) *Lampsilis parva*, four; (4) *Lampsilis gracilis*, three; (5) *Plagiola elegans*, one; (6) *Plagiola donaciformis*, sixteen; (7) *Anodonta imbecillis*, two; (8) *Ptychobranchus phaseolus*, two; (9) unnamed species, one.

The first of these specimens was secured on August 20, 1910, in the Kiamichi River, near Fort Towson, Oklahoma. While working in a gravel bed, Owen Horne, one of our party, called attention to an unusually small mussel attached to pebbles by a byssal thread. After a search for about two hours we secured eight specimens with byssus, representing four species. These specimens were found in a coarse gravel bed, the pebbles being from one fourth to one inch in diameter. The water was fairly swift, and from one to two feet in depth. The byssus of these specimens was variable in length, sometimes several inches long, and often connecting three or four small pebbles. Sometimes the byssus spread into several branches at the place of attachment. The young mussels were best secured by taking up handfuls of gravel and looking for the thread. The byssus is strong enough to support the mussel in a rapid current, and will sustain the weight of a number of small pebbles, without breaking.

On August 30, 1910, five more specimens were secured in the

[1] Most of the specimens have been examined by F. C. Baker and L. S. Frierson, making sure of the identifications.

Little River, near Garvin, Okla. One of these specimens, found by E. C. Johnston, of our party, was attached by the byssus to the shell of a large *Quadrula pustulosa*. An observation of this kind is described by Kirtland.[1]

During the afternoon of August 30, we again investigated a portion of the Kiamichi River, near Roby, Okla. Here we secured fourteen specimens; ten of these I secured in about half an hour, one time bringing up three specimens with a handful of gravel. Here again the environment was typical, fairly swift water, coarse gravel, and rocks.

A search for young mussels was also made in Blue and Boggy rivers, but failed to yield specimens with functional byssus. However, a number of mussels under twelve millimeters were secured, representing *Quadrula pustulosa*, *Quadrula lachrymosa*, *Quadrula coccinea*. In the Washita River, near Davis, Okla., on September 2, we secured the last of our young mussels for the summer. Five specimens were found in swift water about two feet deep. One of these was under three millimeters in length and weighed .005 of a gram.

In the following table I show species, locality, weight in grams, length, height, and breadth in millimeters, of ten of the thirty-two specimens:

Species.	Weight.	Length.	Height.	Breadth.	Locality.
1. *L. luteola*	.41	14.5	9.2	5.9	Kiamichi R., Aug 30, 1910.
2. *L. fallaciosa*....	—	13.5	6	3.4	Kiamichi R., Aug. 20, 1910.
3. *L. parva*	.035	7	3.8	2.3	Kiamichi R., Aug. 20, 1910.
4. *L. gracilis*.....	.47	22.5	9.9	4.1	Little R., Aug. 30, 1910.
5. *A. imbecillis*....	.055	7.1	3.5	1.1	Kiamichi R., Aug. 30, 1910.
6. *P. phaseolus*....	.15	12.4	5.6	2.7	Little R., Aug. 30, 1910.
7. *P. elegans*.....	.175	9.6	6.9	4.5	Kiamichi R., Aug. 30, 1910.
8. *P. donaciformis*.	.005	2.9	1.5	1.1	Washita R., Sept. 2, 1910.
9. *P. donaciformis*.	.01	5.6	3	1.9	Kiamichi R., Aug. 30, 1910.
10. Unnamed sp.	.014	5	3.3	2.3	Little R., Aug. 30, 1910.

The facts noted above are closely related, not only to the ecology of the juvenile mussel, but also to the ecology of the adult.

1. They indicate the conditions essential for the most successful growth and early development of the Unionidæ. This kind of

[1] "Fragments of Natural History," J. P. Kirtland, *American Journal of Science*, Vol. 39, 1840, pp. 166–168.

an environment gives a constant supply of oxygen, and sufficient food; is frequented by suitable fish; is free from shifting sand and silt accumulation. Those mussels that drop from the fish in these favorable situations develop in large numbers, while the less fortunate, that drop in shifting sand and silt, die early.

2. In the study of the ecological factors that are inimical to mussel life, more attention should be given to the consideration of the juvenile habitat. Absence of gravel bars and stony situations may sometimes explain the scarcity of the Unionidæ in certain streams and lakes where frequently water content has been thought the chief unfavorable factor.

3. It is a well known fact that in many streams certain stretches of mud-bottom are found loaded with mussels, while other areas, in the same stream, equally favorable from the standpoint of the habitat of the adult mussels, have only scattering specimens.

This distribution of the adult may be explained by the assumption[1] that the average mussel seldom travels far up or down the stream from the place where it begins successful development. Stretches favorable for juvenile development thus come to be the centers of dispersal in the streams where they occur. As a result, areas of mud-bottom near these favorable habitats, become loaded with mussels by migration.

4. In the study of the life history of the Unionidæ, we may consider the embryonic, the glochidia, the parasitic, the *early juvenile*, and the adult as distinct periods for separate and special study.

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November 25, 1910.

[1] This assumption is fairly well established, by experimental study that I have been carrying on for some time, and will be discussed in a later paper.

A STUDY OF PEDAL LACERATION IN ACTINIANS.

LEWIS R. CARY.

Since the appearance of Andres's ('82) classical paper "Intorno alla Scissiparita della Attinæ," the only accounts of the process of pedal laceration have been very casual, or have been given only for the sake of comparison with the phenomena of induced regeneration. Carlgren ('04), ('09), who alone has studied this form of reproduction in the genus *Aiptasia*, makes mention only of the sequence of the appearance of the mesenteries and tentacles, but gives no general account of the process, nor does he mention any of the histological features.

Since the last-mentioned phase of the subject is of considerable interest as a comparison with the development of normal embryos, it has seemed advisable to publish the following account to supplement the previous contributions to the knowledge of this type of reproduction which is so common among the actinozoa.

The observations herein recorded are based upon the study of four species of actinians: *Aiptasia pallida* Agg., *A. tagetes* D. & M., *A. annulata* Andres and *Cylista leucolena* Agg.[1] Of these species *A. pallida* and *Cylista* were obtained in abundance at Beaufort, N. C.,[2] during the summer of 1903 and 1904, where they were studied both in their natural environment and in aquaria in the laboratory.

Specimens of the first-mentioned species were transferred to Baltimore and kept during two years in aquaria in the zoölogical laboratory of the Johns Hopkins University. By means of the diatom method described by Dr. Caswell Grave for rearing echinoderm larvæ, the actinians were kept in good condition

[1] Professor McMurrich informs me that he has conclusive evidence that the form described by Verrill from Beaufort, N. C., as *C. leucolena* is not the *C. leucolena*, of A. Agassiz, but in the absence of any published statement to that effect Verrill's terminology will be used.

[2] I am indebted to the officials of the U. S. Bureau of Fisheries for the use of a table, and equipment at their Beaufort, N. C., laboratory during the two seasons above mentioned.

during this time and they continued to reproduce by laceration throughout the year, although no embryos were ever set free during either of the breeding seasons.

The specimens of *A. tagetes* studied were obtained in 1909, during a stay at the Bermuda Biological Station.

A. annulata was obtained during the past summer while the writer was at the laboratory of the Carnegie Institution of Washington at Dry Tortugas, Florida.

The two last-mentioned forms were studied in aquaria in the laboratory only.

Material of the four species was preserved, after being narcotized in magnesium sulfate, in a number of fixing reagents; of which Petrunkewitsch's fluid and Bouin's fluid gave the best results. All of the *Aiptasias* cut readily after being imbedded in paraffin in the usual manner, while the mesoglea of the *Cylistas* proved so refractory that double imbedding in celloidin and paraffin was necessary in order to secure an unbroken series of sections.

In common with the tissues of most actinians, many of the hæmatoxylin stains were highly selective, staining the ectoderm much more heavily that the endoderm so that by using an alcoholic solution of eosin as a counter stain after Delafield's hæmatoxylin there resulted an absolutely certain color differentiation between the two tissues by means of which any question of the origin of a certain structure could be determined by its staining reaction.

General Account of the Process of Laceration.

As has been described by Andres (1882), the beginning of the process of laceration is characterized by a certain part of the base of an actinian becoming very firmly attached to the substratum while the animal as a whole moves away from the point which is thus relatively immovably attached. As a result the tissues about this point become strongly stretched and attenuated. With the continuation of the contraction, there finally comes a rupture of the tissues at some little distance from the free border of the disc so that the resulting laceration piece has usually an appearance such as is shown in Fig. 1, Plate I., which represents a fragment from the base of a specimen of *A. pallida* immediately

after its separation from the parent individual. The *acontia*, which in laceration pieces of this form are at first so prominent, are later either withdrawn inside the cavity of the fragment, or else they are broken off before the open side of the piece has closed to any considerable extent.

Immediately after the separation of the fragment there takes place a rolling-in of the free edges, due principally to the elasticity of the mesoglea, so that for some little distance within the cavity is lined with ectoderm (Fig. 7, Pl. II.). For some hours after the separation of the fragment there is no visible external change other than an increasing loss of color about the orifice of the tear where the laceration piece was separated from the parent individual.

The next noticeable change appears as a turning upward of that portion of the fragment in which is situated the original opening, which by this time has become markedly reduced in extent; although, contrary to the results of Hazen (1902), who worked with fragments obtained by cutting off pieces including portions of the column wall and pedal disc from adult specimens of *Sagartia lucæ*, in none of my specimens did the opening become entirely obliterated. The new growth which brings about this change in the shape of a laceration piece is much the more rapid on the lower-pedal disc-side of the aperture. So that for some time the elevated area is situated near the former internal edge of the fragment. The wall that has come from the up-growth from the pedal disc is nearly perpendicular, while the opposite side of the fragment slopes away much as in its former manner (Fig. 2, Pl. I.).

In a stage of development such as is represented in Fig. 2 there has already appeared the beginning of the tentacle buds, and internally, of course, the earliest mesenteries.

In a later stage, as in Fig. 3, Pl. I., the readjustment of materials had proceeded farther in what is practically the same course. The fragment as a whole has become much higher. The oral end has nearly the same diameter, while farther down toward the pedal disc the diameter is relatively much decreased. At the base, however, the fragment still covers practically the same area as when it was torn off from the parent animal. When

viewed from the side, as in this figure, the lines corresponding to the insertion of the old mesenteries are seen to extend only a short distance upward from the base and to be less distinct toward that side which is most nearly perpendicular, *i. e.*, which has arisen from the tissue which was originally part of the pedal disc. The tentacle buds have increased very slightly in size, but at the oral end of the fragment, which now has the appearance of a partly contracted *Aiptasia* there can be made out through the almost transparent column wall, the outlines of the stomodeum and the lines of insertion of the developing mesenteries.

Figs. 4 and 5 were, in order to show the arrangements of the tentacles, drawn in a nearly oral view, so that the upper part of the column is not visible. The fact that the lines of insertion of the old mesenteries appear to extend nearly the whole length of the column is thus to be explained by the foreshortening in the figure and not by any difference as regards the mesenteries between these specimens and that shown in Fig. 3. In the specimen shown in Fig. 4, there are eight tentacles, of which one is considerably longer than the other seven, all of which are of approximately the same length. In practically all instances one tentacle outstrips the others in its development so that for a considerable time it may be distinguished from the others. On the other hand there seems to be no such definiteness in the determination of just what tentacle shall first appear as there is in many actinian embryos where the tentacle above the endocœle between the two dorsal directive mesenteries almost invariably makes its appearance first.

In later stages there is often a very marked difference in the sizes of the tentacles of the first series, as in the specimen represented in Fig. 5. Here a single tentacle has far outstripped the others, while of the remaining seven three are decidedly larger than the remaining four. In Fig. 5 the tentacles of the second set are seen to be arising in pairs at each side of several of those of the first set. While that tentacle of the first set which is uppermost in the figure is by far the longest, there is on either side of it a single short tenacle of the first set, and no indication of the paired tentacles of the second series. The tentacles on the opposite side of the disk have already acquired their definite shape and appearance.

The young actinian shown in Fig. 6 has already acquired the characteristic appearance of all small specimens of this species, so that, from an external examination, it would be impossible to determine whether it had come directly from an egg or from a laceration piece. As compared with any of the figures of earlier stages the proportionate diameter of the column is very much reduced. This has been brought about by a continuation of the same processes that are shown at an earlier stage in Fig. 3. The basal portion of the column is now of no greater diameter than the upper part, while the whole animal has become considerably elongated. The first eight tentacles are now of practically the same length as are also the eight pairs of secondary tentacles. Internally the stomodeum can be seen to have increased greatly in length. The mesenteric filaments, not previously distinguishable, have now become very prominent structures on the first eight mesenteries. The column wall has become thin and has lost its pigment clear to the base through which are seen the points of insertion of the first eight mesenteries, while those mesenteries that came over from the parent actinian in the laceration piece, have entirely disappeared, even at the extreme base of the young specimen.

In following through the development of any single laceration piece from the time of its separation from the parent until it becomes a typical young actinian it is apparent that there has been very little, if any, increase in its bulk, and that the change in shape has come about almost entirely through the readjustment of the materials already present and not to any considerable extent through the metabolism of new material.

Frequency of Reproduction by Laceration among the Forms Studied.

The following table shows the frequency of this method of reproduction among the four species studied and the number of young that has arisen from a single parent individual within a given time.

In another instance many thousand individuals of *A. pallida* were examined casually, but without any records being kept, and here again it was found that the percentage of those which

had undergone laceration was very high. In fact this form of reproduction was much more frequent in the last-mentioned instance—on the government jetties at Cameron, La., than in the case of the same species from which the records were made at Beaufort.

Species.	Number Examined.	Number that had Given Rise to Laceration Pieces.	Number of Laceration Pieces.	Locality.
C. leucolena	300	292	11.32 (0–30)	Beaufort, 1903.
C. leucolena	1,000	942		Beaufort, 1904.
A. pallida	150	78	6 (0–14)	Beaufort, 1904.
A. tagetes	220	187	10.4 (0–26)	Bermuda, 1909.
A. annulata	52	20	3 (0–7)	Tortugas, 1910.

Internal Changes During Laceration.

Under this head will be discussed the following topics:

(*a*) The formation of the stomodeum.

(*b*) The development of the mesenteries.

(*c*) The development of the mesenteric filaments and acontia.

(*d*) General histological changes in the tissues during development.

(*a*) *The Formation of the Stomodeum.*

As mentioned previously in the general account of the process of laceration, the first noticeable change in the appearance of a laceration piece after its separation from the parent consists in the rolling in of the edges of the torn side of the fragment, so that from the first the opening into the cavity of the fragment is lined for some distance with ectoderm (Fig. 7, Pl. II.). At first the ectoderm extends forward for only a short distance on the upper side of the opening, while on the lower side the inrolling has progressed considerably farther.

In a section through a stage such as is shown in Fig. 2, Pl. I., the stomodeum has the appearance such as is shown in Fig. 8, Pl. II. Here the free edges about the orifice of the fragment have become thinner, the mesoglea is reduced to a thin sheet of tissue and the cavity of the stomodeum has become vertical instead of nearly horizontal as at first. At the lower, free, border of the stomodeum the ectoderm has become turned outward and extends around the border of the endoderm onto the internal wall of the stomodeum.

In Fig. 9, Pl. II., which represents a section through a stage slightly older than that shown in Fig. 3, Pl. I., the stomodeum has reached nearly its normal length and, on the left side in the figure, its ectodermal lining is shown extending down along the border of a mesentery to form the mesenteric filament. On the opposite side where the section passes through the chamber between mesenteries, the ectoderm forms a flat plate extending across the free border of the stomodeum.

(*b*) *The Development of the Mesenteries.*

Andres (*loc. cit.*), in his figures illustrating the rearrangement of the mesenteries in the young fragments, shows only the pedal disc and apparently understood that the mesenteries which come over in the laceration piece from the parent were rearranged to become the permanant mesenteries of the young actinian. He makes no mention of any new growth of mesenteries or stomodeum at the oral end of the fragment as it becomes elongated and assumes the characteristic shape of an actinian.

Carlgren, 1904, 1909, takes up to a considerable length the question of the arrangement of the mesenteries in laceration embryos. He distinguishes four types of arrangement of the first twelve mesenteries in such embryos: depending both on the sequence in the appearance of the mesenteries, and on whether or not any of these mesenteries, are situated in old tissue. "Was erstens die Entwicklungstypus der Mesenterien in den Lacerationstückchen anbelangt, so haben die 1904 und die hier oben mit geteilten Untersuchungen gezeugt dass es verschiedene Variationen derselben giebt. Wie gross die Variation auch ist, so kann mann doch deutlischerweise in dem Lacerationstückchen, d. h. in solchen Stückchen, die einen Teil der Fussscheibe und einen der Korperwand umfassen (Text Fig. 1, *b*), drei Verschiedene Haupttypen für die Mesenterien Entstehung unterscheiden, und zwar erstens einen *bilateralen* mit *drei* primären, vollständigen, gleich, orientierten Mesenterienpaaren (II., 4.), Zweitens ein *bilateralen* mit nur *zwei* ebenfalls gleich (II., 1), angeordneten Paaren und schliesslich drittens einen *birädialen* (II., 5 and 8), welche letzteren zwei Neubildungszonen enthält, die in ihren am besten entwickelten Gestalt (No. 8), Spiegelbilde zu einander

sind, indem jede aus zwei bilateral angeordneten, vollständigen Mesenterienpaaren besteht." Besides these three types Carlgren recognizes a mixed type which cannot be put in any one of the above-mentioned classes.

All the specimens of *Aiptasia* studied by Carlgren fell, with three exceptions which were very irregular in their growth, into his group three, the biradial type, in which there are two zones of new growth. All of the specimens of this genus that I have sectioned show this arrangement of the newly formed mesenteries, which, thus, seems to be characteristic of the genus *Aiptasia*.

The specimens of *Cylista* also showed the same arrangement of the first mesenteries.

H. V. Wilson, 1888, states that in *Macina areolata* the œsophageal invagination is at first centrally placed in the larva, but that soon "it begins to travel toward one side of the larva." This lateral motion of the stomodeum continues until that organ comes to lie close against the outer wall of the larva. Finally as the lateral movement progresses—from above downward—the endoderm is pushed before it until the two ectodermal layers, of the stomodeum and of the body wall, are in contact, or separated only by the thin layer of mesoglea secreted between them. Soon following this stage, . . . "the œsophagus has moved away from the surface ectoderm, but while doing so has remained connected with it by a band of supporting lamella." The mesentery arises by the endoderm growing up into the space between the stomodeum and the outer wall of ectoderm as the stomodeum is drawn toward the side opposite the first mesentery, for the formation of the second one.

Appellöf, 1900, mentions the same sort of displacement of the stomodeum previous to the formation of the first mesentery. He states, on the other hand, that: "Die exzentrische Lage des Schlundrohres ist in allen Entstehungsphasen desselben konstant und deutlich nachzuweisen und ist—so nehme ich wenigstens an—auf ungleichseitiges Wachstum der Larve zurückzuführen."

In laceration embryos the stomodeum is from its first appearance excentric in position. As the change in the form of the piece takes place so that the original orifice, where the piece was torn off from the parent, becomes carried up toward the highest

part of the fragment and becomes smaller and more rounded, the stomodeum on that side which has come from the growth of the pedal disc comes into contact with the body wall. The first mesentery makes its appearance at the point where the forming stomodeum is in contact with the column wall; although in none of my sections is there evidence of any marked displacement of the endoderm such as would be necessary to bring the two mesogleal surfaces into contact. As is always true in the formation of a mesentery, the growth of the mesogleal lamella here begins at the extreme oral surface and proceeds downward along the stomodeum and column wall. In laceration embryos the endoderm is carried down on both sides of the mesogleal lamella so that from its earliest appearance, the mesentery is structurally complete. As the growth leading to the acquisition of the characteristic actinian shape of the laceration piece goes on the stomodeum becomes situated more centrally in the oral disc. While this adjustment is taking place the first mesentery becomes lengthened in [the horizontal [direction to keep pace with the change in the position of the stomodeum.

The method of the formation of all those mesenteries formed after the stomodeum has become central in position is the same. The first indication of their appearance is seen as a slight ridge of mesoglea extending aborally from the oral disc at the point where the latter joins the column wall. As the growth of the mesentery continues the mesogleal lamella extends farther from its point of origin, both horizontally along the oral disc and proximally along the column wall. When by its horizontal growth the mesogleal lamella comes in contact with the mesoglea of the stomodeum a rapid downgrowth of the mesentery takes place. Throughout the development of a mesentery the growth is the most rapid where there is contact between the growing mesogleal lamella and that of the body wall, oral disc, column wall or pedal disc. In all such mesenteries the growth of the endoderm keeps pace with that of the mesogleal lamella so that the latter never extends to the free border of the mesentery.

In Figs. 10 to 14, Pls. II. and III., are shown a number of sections through the same laceration embryo at different levels. In Fig. 10, which passes close to the oral disc, only four complete

mesenteries are present while a member of the third pair has made its appearance on one side of the longitudinal axis of the stomodeum.

In Fig. 11, a section taken through the embryo near the base of the stomodeum, seven mesenteries are present. The same four as noted in the previous section are complete while both those of the third pair are present and one of the fourth pair is seen as a slight prominence of the endoderm containing a very thin mesogleal fold. In Fig. 12 only six mesenteries are present, but it seems that one of the third pair is lacking instead of the single one of the fourth pair which was seen in the previous section.

The first pair have already acquired their mesenteric filaments, which at this stage extend only a short distance below the stomodeum.

Farther down, at the level of the section shown in Fig. 13, only four new mesenteries are present, while seven of those which came over in the laceration piece from the parent are seen. This last-mentioned section was taken about one third of the distance from the oral disc to the base of the embryo, and in this series the newly formed mesenteries are found in only four of the following sections.

In the sections shown in Fig. 14, all of the mesenteries present are those which were in the laceration piece at the time when it was separated from the parent. Fig. 14, although taken at some distance from the base shows, in comparison with the previous sections, that the basal portion of the embryo is of considerably greater diameter than the more distal portions.

A comparison of the three last-mentioned figures illustrates one phase of the redistribution of materials in the growth of a laceration embryo. In Fig. 13, that side where the new mesenteries are being formed is very thin-walled, the mesoglea having become reduced to a thin sheet throughout that half of the body. In the remaining half, on the other hand, the tissue relations of an adult *Aiptasia* are still maintained. In the sections through the more proximal part of the body the adult tissue relations are apparently undisturbed, at least morphologically. It will be seen, however, that in this region there has been coincident with the elevation of the oral end of the embryo a drawing together of

the basal portion so that it is now nearly circular in outline. In Fig. 15 it may be seen that the old mesenteries which formerly occupied only one side of the fragment—at the time of its separation from the parent—have now come to be distributed so that they now arise from about four fifths of the circumference.

In sections through an older specimen, as shown in the series of figures from Fig. 15 to Fig. 18, the second set of mesenteries has made its appearance about the inner surface of the oral disc, and eighteen of them have reached the stomodeum. Farther down along the stomodeum (Fig. 17), the twelve primary mesenteries are complete while only one other pair has made sufficient downward growth to appear in such a section.

In Fig. 18, where eleven of the twelve primary mesenteries are present, none of those which came over in the laceration piece from the parent animal are in evidence. Eight of the new mesenteries have acquired their filaments, but only those on the first pair of mesenteries are trilobed.

As a basis for the comparison of this series with the previous one attention may be called to the appearance of the mesoglea in Fig. 18. At a corresponding height on the column of the individual shown in the former series (Fig. 14), none of the newly formed mesenteries were present, while in Fig. 13, where only four of them had appeared, that side of the column on which they were situated showed all of the characteristics of newly formed tissue.

This shows beyond question that in the older specimen the new mesenteries have extended down to a part of the body where there were formerly some of the older mesenteries which by this time have been entirely resorbed. It will be noticed that in this part of the body the tissue readjustment has proceeded until the relation between the layers characteristic for the adult has been reached.

Carlgren, 1904, in discussing a series of sections through an embryo in which eight of the new mesenteries had reached the stomodeum figures two sections and makes statements from which I can infer only that he understands, either that some of the old mesenteries are brought into the permanent systen of mesenteries of the laceration embryo, or that some of the new mesen-

teries arise at a level below the lower end of the stomodeum. Either of these conclusions is at variance with the results which I have obtained in the study of the four species of actinians on which the present paper is based.

Carlgren's figures and the statements directly relating to them are as follows:

Ein querschnitt in der Schlundrohrsregion (Fig. 4, Taf. IX.) zeigt ein birädial Anordnung der vollständigen Mesenterien mit den beiden Enden des spaltförmigen Schlundrohrs sind zwei Richtungsmesenterienpaare vereinigt. An jede Seite derselben liegen zwei vollständige Mesenterien (*a*) ich nenne sie später kürzlich seitliche Mesenterien—die ihre Längsmuskelen gegen einander kehren. In allem sind also 8 Mesenterien vollständig.

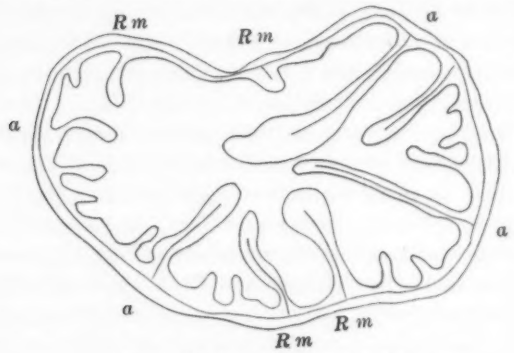

FIG. 1. (Carlgren's Fig. 4, Taf. IX.)

(Wenn ich unter von einer birädialen Anordnung sprache, sind die vollstandigen Mesenterien wie hier grupiert.) Zwischen den Seitlichen Mesenterien an jeder Seite sind verscheidene, unvollständige Mesenterien entstanden, die in dem proximal körpertheilen (Fig. 7, Taf. IX.), ziemlich staak sind; in den distalen an der einen Seite verschwinden, an der anderen nur unbedeutend entwickelt sind oder ganz fehlend (Fig. 4, Taf. IX.). Die mit (*b*) bezeichneten Mesenterien bilden mit ihren Partneren an je ein Mesenterienpaar mit zugewandten Langsmuskeln. Die Mesenterien des Stückchens sind also nach der Sechszahlange-ordenet, obgleich von dem ersten Cyklus vier Mesenterien unvollständig sind. Die zwischen den Mesenterien (*b*), liegenden

Mesenterien gehören einem zweiten Cyklus an. An jeder Seite der Richtungsmesenterien findet man auch unvollständige Mesenterien eines zweiten Cyklus. Auch einige Mesenterien der dritten Ordnung sind angedeutet (Fig. 7, Taf. IX.). An

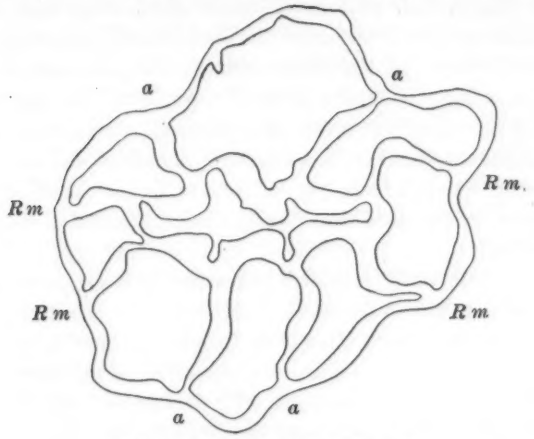

FIG. 2. (Carlgren's Fig. 7, Taf. IX.)

der Bases sind die mit (*a*) bezeichneten Mesenterien am Stärksten, die Mesenterien (*a*) an der rechten Seite der Sagittalachse laufen fast mit den entsprechenden Mesenterien an der anderen zusammen (Fig. 7, Taf. IX.).

His Fig. 4, Taf. IX., my text Fig. 2, shows a condition of the mesenteries which is common to all laceration embryos in a corresponding stage of development. His Fig. 7, Taf. IX., text Fig. 3, shows twenty-five mesenteries, eight of which he considers to be of the first cycle (mesenteries marked *a*) while of the remaining seventeen six are according to his statement of the second cycle (marked *b*) and the remainder of a third cycle. It will be noticed that several of the mesenteries in this figure—one pair of those marked *Rm* and the longest ones at one end of the figure—are very broad in section with a thick mass of endoderm extending far beyond the mesoglea. None of the entire number of mesenteries have any trace of mesenteric filaments, nor by their structure, in so far as it can be made out from the original figures, is there any indication that they contain actively

growing tissue as is always found in the new mesenteries in the forms that I have studied. Comparing these two figures with those of the series shown in my Figs. 10 to 14, Pls. II. and III., it seems very evident that all of the mesenteries shown in Carlgren's Fig. 7, Taf. IX., are old ones which have come over in the fragment from the parent individual and which will never come to be a part of the permanent system of mesenteries of the actinian arising from the laceration embryo. In the younger embryo shown in my series of figures just mentioned the first formed pair of mesenteries has already acquired their mesenteric filaments, which are as yet, of course, in an undifferentiated condition; but nevertheless clearly distinguishable as mesenteric filaments.

In the embryo from which my series of Figs. 16 to 18 was taken, which has at the level of the stomodeum only four more mesenteries than that from which Carlgren's figures under discussion were taken, eight of the new mesenteries, at the level at which Fig. 18 was taken, are provided with filaments of which those on the first pair of mesenteries are already trilobed, while several of the others show the first steps in the process of differentiation by which the trilobed condition is reached.

(*c*) *The Development of the Mesenteric Filaments.*

The question of the origin of the mesenteric filaments among the anthozoa has been discussed to considerable length by most workers on the development of these forms. With the exception of E. B. Wilson, 1884, all of the previous investigators have confined themselves to a consideration of the embryonic development. The above-named investigator worked with the polyps arising by budding in several alcyonarians, but not with embryos of the same forms.

E. B. Wilson (*loc. cit.*) reached the conclusion that in the Alcyonaria the dorsal directive filaments arise as downgrowths of the ectoderm of the stomodeum, while all the other filaments arise through the differentiation of the endoderm at the free border of the mesentery. He would homologize the dorsal filaments of the Alcyonaria with the lateral ciliated bands (flimmerstriefen) of the trilobed actinian filament. The filaments

of the remaining mesenteries he would homologize with the glandular streak (Nesseldrüsenstreif) of the trilobed filament. Thus, according to his interpretation, the actinian filament is a double structure.

H. V. Wilson (*loc. cit.*) working on the coral *Manicina*, where the filament consists of a single lobe, reached the conclusion that this type of filament was comparable to the trilobed actinian filament and is entirely of ectodermic origin.

McMurrich, 1891, describes a double origin for the trilobed filaments of *Rhodactis* and *Aulactinia;* after the manner suggested by E. B. Wilson.

Duerden, 1899, working on *Lebrunia*, where the mesenteries become developed before the stomodeum becomes hollowed out, concluded that in this case part, at least, of the filaments are of endodermic origin since he finds stages where filaments are present on some of the mesenteries which as yet have no direct connection with the stomodeum.

Appellöf (*loc. cit.*) concludes that the mesenteric filaments are developed entirely from the ectodermal downgrowth from the lining of the stomodeum. He mentions having observed a number of instances where the ectodermal layer had become very much attenuated just below the level of the stomodeum, and indeed in some instances the continuity of this layer had been broken. He argues from this fact that some of the instances observed by previous workers who, on the basis of a lack of continuity between the mesenteric filament and the lining of the stomodeum, maintained the endodermic origin of the filaments might be simply cases where the continuity of the tissue had been broken and in no way fundamentally different from the ordinary course of events as he has described them. He also makes the suggestion that the method of development described by E. B. Wilson for the alcyonarian filaments may be confined to those individuals which arise as buds and not a true explanation of what takes place during embryonic development, which he tacitly assumes would be similar to plans followed in other Anthozoan forms.

In all descriptions of the formation of the mesenteries, and particularly of the mesenteric filaments, except that of Duerden

(*loc. cit.*) for *Lebrunia*, it is recorded that the filaments appear first on the upper part of the mesenteries at the level of the free border of the stomodeum, and usually there can be observed a direct continuity between the ectodermal lining of the stomodeum and the filaments from the first appearance of the latter.

The mesenteries acquire their filaments in the same order as that in which they arose, so that the dorso-laterals would first show these structures. In Fig. 12, Pl. II., this pair of mesenteries alone shows the beginning of the filaments. Farther up in this series of sections the tissue forming the filaments is directly continued into the lining of the stomodeum. In Fig. 9, Pl. II., a longitudinal section through a young laceration embryo, the filament extends for a short distance down along the free border of the mesentery, and here there is no histological differentiation to mark the lower limits of the stomodeum. Its actual limit can, however, be determined by a comparison with the other side of the figure where the section passed between two mesenteries so that the border of the stomodeum hangs free in the gastrocœl. H. V. Wilson (*loc. cit.*) has pointed out that in *Manicina* the filaments of all the first twelve mesenteries, save the first pair, arise from a fold of the ectoderm at the free border of the stomodeum which bends up and outward into the gastrocœl pointing toward the oral disc. In this manner the filament begins to grow down the mesentery before the edge of the latter has in its upper part come into contact with the stomodeum.

In the development of laceration pieces of *Aiptasia* there is the same folding backward of the wall of the stomodeum at an early stage in the development (Fig. 8), so that the course of the development of the filaments in this form follows that already described for *Manicina*.

In an older specimen, Fig. 18, Pl. II., the filaments of the first pair of mesenteries have assumed the trilobed form characteristic of the filaments of adult *Aiptasias*, while the remainder of the mesenteries have at this level either simple filaments or none at all. It is thus apparent that the change in the structure of the filaments follows the same order as does the appearance of the filaments themselves.

In this transition from the simple to the tri-lobed structure

there is in these forms no indication of the origin of the ciliated bands as an acquisition of new or different tissues, as McMurrich has suggested in arguing for a dual origin for the parts of the complex filament, but, on the other hand, the lateral lobes of the complex filament arise by a very slight differentiation of the cells already present in the filament from the time of its origin from the stomodeal ectoderm. In Fig. 17, Pl. III., the filaments of the dorsal directive, the ventro-lateral and the ventral directive mesenteries all show in their centers the beginning of the growth which will give to the mesogleal layer its characteristic branched appearance. Each of these branches will become the center and support, of one lobe of the adult trefoil filament. In the dorsal directive mesenteries especially, it may be noticed that the tissues of the filament have extended out around the edges of the mesentery so that the filament is nearly circular in outline. Originally the extension of the stomodeal ectoderm—first appearance of the filament—was in the form of a flat sheet (Fig. 12, Pl. II.). The separation of this sheet of tissue into the three lobes of the adult filament apparently takes place through an unequal growth from three points in the nearly circular filament, as shown in Fig. 18, Pl. III.

As may be seen by comparing Fig. 9 and Fig. 12, Pl. II., with the fully formed filament (*e. g.*, the filaments of the dorso-lateral mesenteries in Fig. 17, Pl. III.) the histological changes which have taken place in the transformation of the "embryonic" tissue of the stomodeal ectoderm to those of the tri-lobed filament have been in the nature of a separation of the cellular elements of the original tissue so that one type of cells (ciliated) is centralized in the lateral lobes; while the other types (gland and nettle cells) are concentrated in the median lobe. The apparent change is greatest in the case of the median lobe, since the undifferentiated stomodeal ectoderm is at the time of the first appearance of the filaments poor in gland cells and nematocysts. This last-mentioned fact is also true of the activity growing ectoderm of the new part of the column wall and oral disc, and in all parts of the body there is a marked increase in the actual as well as in the proportionate number of both these types of cellular elements after the normal body proportions have been established.

(*d*) *Histological Changes During Development by Laceration.*

In considering the histological changes undergone by the tissues during development by pedal laceration those changes which take place in the basal portions of the parent individual before the fragment is torn off may be first mentioned. Andres (*loc. cit.*) mentions that in *Aiptasia lacerata* the rim of the basal portion of the animal becomes very opaque, and that in section there seems to be an unusual number of gland cells in this region. The endoderm becomes very much thickened in this region so that when the fragment becomes torn off there is only a small cavity left in its interior (Fig. 7, Pl. II.). As may be seen in this section, the thickening of the endoderm is very much greater on the upper side of the fragment, *i. e.*, that which has come from the original column well.

In the period immediately following upon the separation of the fragment from the parent, there is a rapid change in the character of the tissues about the orifice. All of the newly forming tissue becomes very light in color due to the comparative paucity of the zoöxanthellæ in the endoderm of the active area. The mesoglea becomes comparatively thin in this region, but seems always to arise as a direct prolongation of that already present and not as a new secretion between the ectoderm and endoderm of the new parts.

In the cellular layers the changes are more profound. In the ectoderm (Fig. 19, Pl. IV.), a section through a young tentacle bud, the boundaries of all of the cells except the nematocysts and gland cells becomes lost so that the whole layer constitutes a syncytium. The cytoplasm is uniformly granular throughout. The nuclei are scattered through the cytoplasm, but still indicate by their positions the fact that only definite cell walls are wanting in order that the usual cell structure should be apparent. At the internal boundary of the entoderm there is distinguishable a layer of very fine muscle fibers. When compared with the size of the nuclei and nematocyst cells, these fibers are seen to be several times less than the diameter of normal muscle fibers of this species of actinian. Since in the fully developed *Aiptasia* muscle fibers exist both as processes from epithelio-muscle cells and as nearly the entire component of "muscle cells" in which the cytoplasmic cell body is reduced to a small mass containing

the nucleus, it is impossible in this case to determine whether we have to do with fibers of the first or of the second of the two above-mentioned kinds. In other words the fibers in question may be in the process of becoming differentiated from epithelial cells, or they may be arising through the division of previously existing muscle cells of the second type. At this stage there is no indication of interstitial cells or of developing gland cells in the ectoderm.

In the endodermal layer of the same section there is exhibited a similar condition of the tissue: Cell walls are entirely wanting; the nuclei, however, by their position indicate a potential cellular arrangement of the cytoplasm. A particularly striking feature as regards this layer is brought out by a comparison of the figure under discussion and Fig. 7, Pl. II. In the last-mentioned figure the endoderm, throughout most of its extent, is considerably thicker than the ectoderm, while in the new tissue the former is generally so thin that the single zoöxanthellæ are greater in diameter than the layer of cytoplasm making up the endoderm and consequently they cause a prominence extending into the cavity of the tentacle wherever they are situated. In some few instances the zoöxanthellæ are placed one above the other, and n such cases there is either a generally thickened area of the cytoplasm or else a cylindrical prominence, as is seen on one side of the figure. All cellular differentiation is entirely lacking in the endoderm. At no place about the circumference of this layer is there any indication of the layer of endodermal (circular) muscles, which are in normal *Aiptasia* tissues, when seen in transverse sections of the animal, much more prominent than the ectodermal muscles.

At the base of the fragment where the rearrangement of the materials is proceeding at a relatively slow rate there seems never to be a breaking down of the tissues comparable to that just described. The mesogleal layer becomes thinned out progressively so that at first it is of its usual thickness on one side of the fragment—that side originally opposite the point where the fragment was torn off from the parent animal—while on the opposite, more actively growing, side it has become relatively thin (Fig. 13, Pl. III.). The cellular layers retain practically their original characteristic appearance, although, as is shown in Fig. 15,

Pl. III., the ectoderm on the side where the new growth is the most rapid, *i. e.*, where there are no old mesenteries in the figure, is much lower than on the opposite side.

When in the later development the cellular elements of the ectoderm and endoderm become delimited by the appearance of cell walls it is noticeable that all of the cells show at first an embryonic character in that they are relatively wide in proportion to their height (compare Figs. 10 and 11 with the old part of the tissue in Fig. 14). In the ectoderm of the column wall in Figs. 10 and 11, and more especially in the lining of the stomodeum of this individual, there is a very marked paucity of the nematocysts and gland cells, so that it is apparent that there has not been an increase in these cellular elements to keep pace with the increase in the epithelial cells. In the older embryo from which the series of figures from 15 to 18 was taken the nematocysts and gland cells have reached nearly their normal number and distribution throughout the newly formed tissues.

In the sections shown in Figs. 10 and 11 the endoderm cells are in general broader than the ectoderm cells and not generally of greater height. In the older tissue (Fig. 18) there are no apparent cell boundaries in the endoderm and the zoöxanthellæ are much more numerous.

Summary.

1. The change in the form of a laceration piece, leading up to the acquisition of the typical actinian shape, takes place through the upgrowth of the tissue about the orifice where it was torn off from the parent.

2. The permanent mesenteries arise as new growths in the undifferentiated tissues of the oral end of the laceration piece.

3. The first twelve mesenteries do not appear in the sequence followed by those in egg embryos.

4. As development goes on the old mesenteries—those brought over from the parent when the fragment was torn off—become restricted to a proportionately shorter and shorter part of the base of the young actinian until they are finally entirely resorbed.

5. The mesenteric filaments are formed, just as in egg embryos, from a downgrowth of the ectodermal lining of the stomodeum. Their trilobed condition arises through the differentiation of this tissue.

6. The tissues of the most actively growing part of a laceration piece become very thin; the ectoderm and the entoderm lose all apparent cell boundaries; the mesoglea arises as a direct continuation of that present in the older tissues.

7. The newly formed tissues contain a very small number of gland cells and nematocysts. These two types of cells are developed to the usual number after the tissue relations have bebecome stable.

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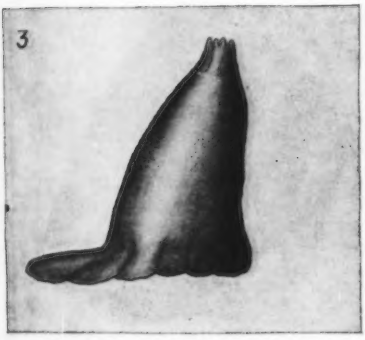
Explanation of Plates.

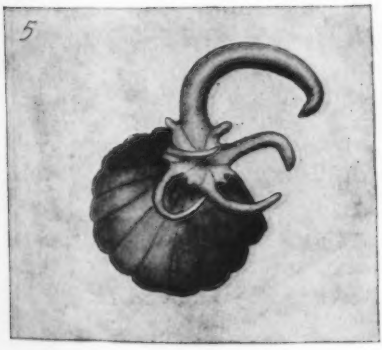
All the figures were drawn from specimens of *A. pallida;* those of the entire laceration pieces from specimens grown in the laboratory; the sections from specimens obtained at Beaufort, N. C.

The ectoderm is stippled in all the figures, the endoderm is distinguished by the presence of the zoöxanthellæ, the mesoglea has been represented by either a single line in the actively growing parts of the embryo or by a clear space between the cellular layers in the older tissues.

Plate I.

The figures on this plate represent a series of stages in the development of a laceration piece, from the time of its separation from the parent until it has assumed the form of a typical young actinian.





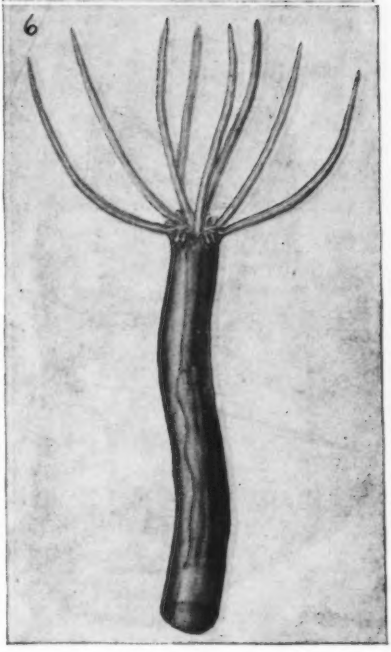


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PLATE II.

FIG. 7. A section through a laceration piece soon after its separation from the parent.

FIG. 8. Part of a vertical section through a young bud to show the formation of the stomodeum.

FIG. 9. Part of a longitudinal section through a young laceration embryo, such as is shown in Fig. 4, Plate I., to show the downgrowth of the ectodermal lining of the stomodeum to form the mesenteric filaments.

FIGS. 10, 11, 12 (with FIGS. 13 and 14, PLATE III.). A series of sections at different levels taken through the same embryo to show the relation of the new to the old mesenteries. (The orientation is the same in all the figures.)

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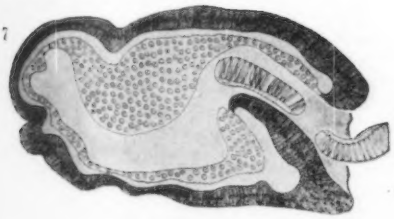

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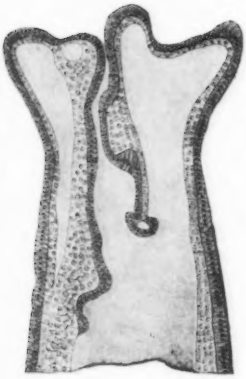

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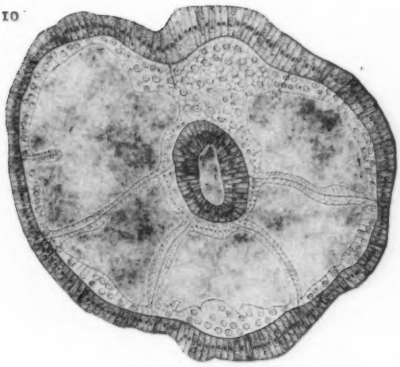

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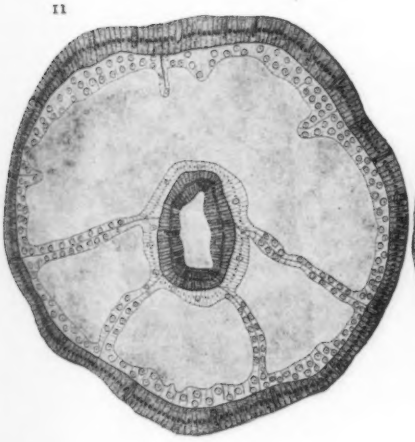

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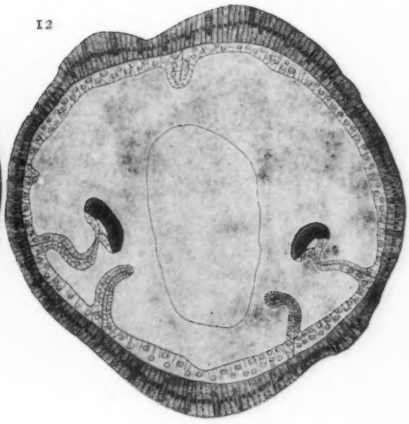

PLATE III.

FIGS. 13 and 14 are part of the series described in the last paragraph. They complete the series which began with Fig. 10.

FIGS. 15, 16, 17 and 18. A series of sections, at different levels, through an older embryo in which all of the twelve primary mesenteries have reached the stomodeum.

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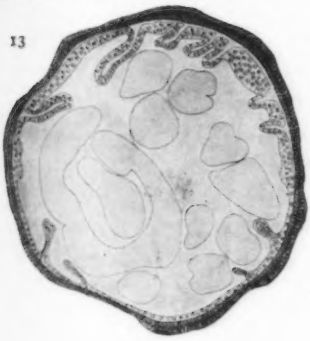

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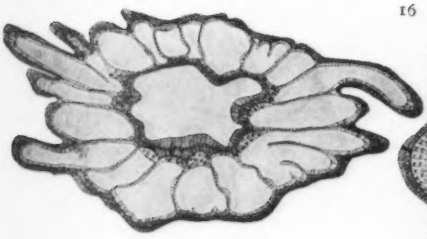

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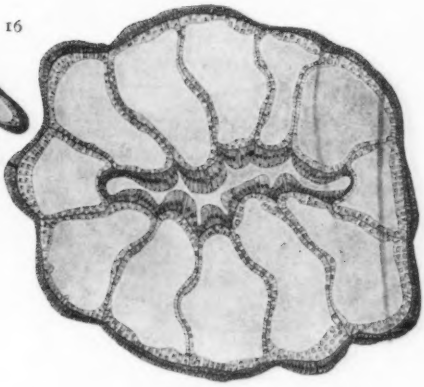

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PLATE IV.

FIG. 19. A section through a very young tentacle bud to show the characteristics of the tissues in an actively growing region of the developing laceration embryo.

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FURTHER STUDIES ON HETEROCHROMOSOMES IN MOSQUITOES.

N. M. STEVENS.

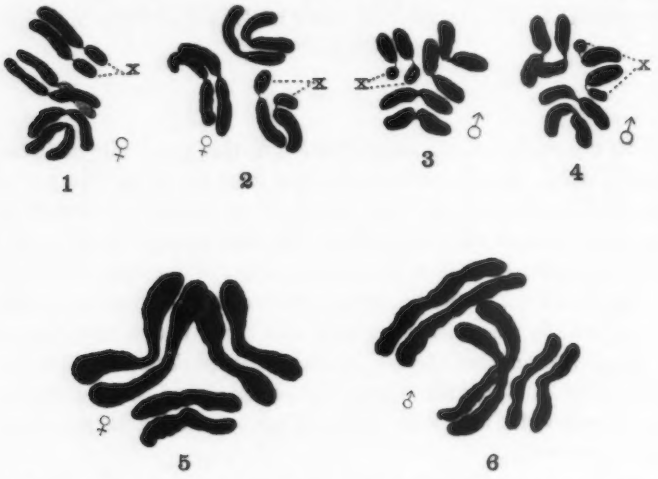
In my study on the chromosomes in the germ cells of *Culex*, published in March, 1910 ('10), there were three figures (11, 20 and 21) which differed from all others for *Culex*, in showing an unequal pair of chromosomes in the spermatogonia (Fig. 11), and a condensed pair in the growth stage of the spermatocytes (Figs. 20 and 21). At the time when the proof of that paper was corrected, it was suspected that the three figures referred to belonged to another species, probably *Anopheles punctipennis*, a few larvæ of *Anopheles* having, it was supposed, been collected in the net with *Culex*, and having escaped detection in both larva and pupa stage.

In October of this year (1910) an abundant supply of *Anopheles* larvæ was secured and a satisfactory study of both male and female germ cells made, showing without question that in the male *Anopheles punctipennis* there is present an unequal pair of heterochromosomes, attached to one of the equal pairs, but otherwise exactly comparable to the unequal pair described by the author for nine species of Muscidæ ('08). A careful reëxamination of the germ cells of *Culex pipiens*, and a study of *Culex tarsalis* and *Theobaldia incidens* in California during the summer, revealed no such heterochromosomes in those species. A description of the chromosomes in the germ cells of *Anopheles punctipennis* and *Theobaldia incidens* will be given in the following pages.

Methods.—The germ glands of both *Anopheles* and *Theobaldia* were studied both in acetocarmine preparations and in sections of material fixed in Flemming and stained with iron-hæmatoxylin or thionin. The latter stain gave very satisfactory results with this material, and saved much time in preparing the sections for study.

Anopheles punctipennis.

Very clear oögonial plates were found in several ovaries, and two of these are shown in Figs. 1 and 2. There are two equal



FIGS. 1 and 2. *Anopheles punctipennis*, chromosomes of the oögonia. Mag. 2,000.

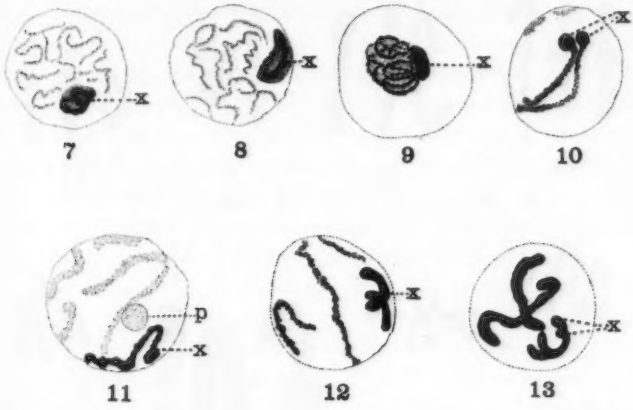
FIGS. 3 and 4. *Anopheles punctipinnis*, chromosomes of the spermatogonia. Same mag.

FIG. 5. *Culex pipiens*, chromosomes of an oögonium. Same mag.

FIG. 6. *Culex pipiens*, chromosomes of a spermatogonium. Same mag.

pairs of chromosomes very similar to those found in other Diptera, and a third pair which is clearly made up of a longer and a shorter pair. Corresponding figures of spermatogonial plates are shown in Figs. 3 and 4. Here the composite pair consists of a longer equal pair, as in the oögonia, united with a smaller unequal pair, the larger member of which is of the same relative size as the members of the corresponding small pair in the oögonial plates. All of the spermatogonial plates in this *Anopheles* material were of this type. In *Culex*, on the other hand, no such inequality or composite condition of one pair of chromosomes could be detected. Figs. 5 (♀) and 6 (♂) are new figures of the oögonial and spermatogonial chromosomes of *Culex pipiens*, and those of *Culex tarsalis* are of the same general character.

In resting spermatogonia of *Anopheles* (Figs. 7 and 8) a condensed mass of chromatin varying in form is found associated with a large plasmosome near the nuclear membrane. In *Culex* there is nothing of the kind—only a large central plasmosome surrounded by an even spireme. In *Anopheles* there is a very conspicuous contraction or synizesis stage (Fig. 9) in which the



FIGS. 7 and 8. *Anopheles punctipennis*, resting spermatogonia. *X* = the heterochromosome.

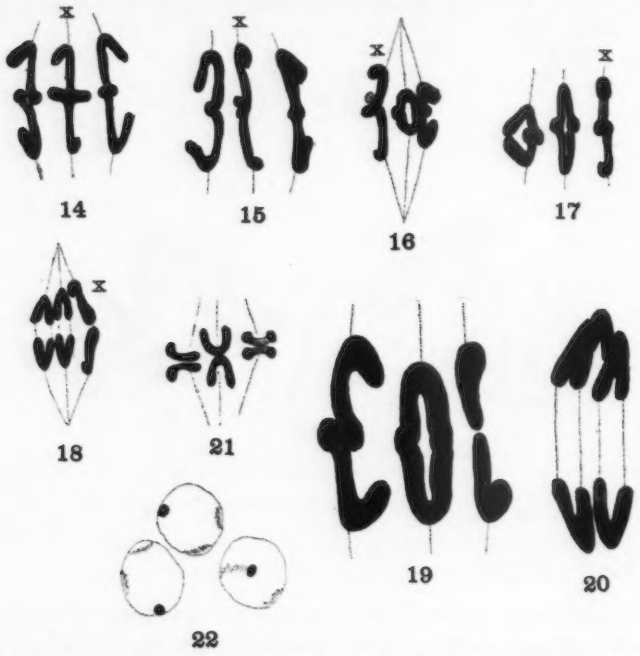
FIG. 9. Synizesis stage.

FIGS. 10–12. Growth stages.

FIG. 13. Prophase.

heterochromosome appears as a deeply staining mass of chromatin resting against a ball of paler chromatin threads. The corresponding stage in *Culex* ('10, Figs. 12 and 13) showed a ball of chromatin thread wound about a plasmosome. In the later growth stages of the spermatocytes of *Anopheles*, the heterochromosome pair assumes a great diversity of forms. Occasionally, especially in early stages, the unequal ends of the pair only are fully condensed as shown in Fig. 10, which is a tangential section of the nucleus. Fig. 11 shows a late spireme stage with heterochromosome (*x*), plasmosome (*p*) and parts of a pale spireme. Fig. 12 is a late growth stage taken from a cyst where the cells were mostly in metaphase of the first spermatocyte mitosis; here the heterochromosome pair shows nothing of its composite

nature, and the members of the pair are in about the position which they assume in the spindle. Throughout the growth stage the composite pair of chromosomes is found, more or less condensed, but always distinguishable from the remainder of the chromatin—against the nuclear membrane, twisted or coiled or



FIGS. 14–17. *Anopheles punctipennis*, first spermatocyte metaphase. *X* = the unequal pair.

FIG. 18. Anaphase.

FIGS. 19 and 20. *Culex pipiens*, first spermatocyte metaphase and anaphase.

FIG. 21. *Anopheles punctipennis*, second spermatocyte metaphase.

FIG. 22. Spermatid nuclei. All figures same magnification—2,000.

knotted into such shapes that it is usually impossible to distinguish the equal and unequal pairs of which it is composed.

Prophase stages are evidently much briefer than in *Culex*, for it was difficult to find any clear figures—Fig. 13 shows the composite heterochromosome pair (*x*) and the other two chromosomes not yet separated into their parallel components.

The character of the chromosome pairs as they appear in the first maturation mitosis is well shown in Figs. 14–17, where the chromosomes are represented in one plane for the sake of clearness. In each figure x is the composite pair. The spindle fibers appear to be attached at the meeting point of the smaller and larger components, the smaller, unequal pair forming the outer ends of the figure and appearing as a conspicuous hook at one end and a rounded knob at the other. No distinct separation of the members of the composite pair, such as is always seen in the spermatogonia (Figs. 3 and 4) was ever observed in the spermatocyte metaphase. The other two chromosomes frequently assume the form of rings in the early metaphase (Figs. 16 and 17) and in the anaphase (Fig. 18) they separate as V's with equal arms, while the heterochromosomes form a pair of hooks with equal long arms and unequal short arms (Fig. 18).

Fig. 19 shows the chromosomes of a first spermatocyte metaphase of *Culex pipiens* and Fig. 20 an anaphase. The chromosomes correspond very closely in form and relative size to those of *Anopheles* with the exception that all three pairs are equal. Both cells and chromosomes are much larger in *Culex* than in *Anopheles*.

In the second spermatocytes the chromosomes are already divided when they come into the spindle and are usually so tangled as to defy any attempt to distinguish the two classes or make clear drawings. In Fig. 21 we have an unusually clear metaphase. An aggregation of chromatin, apparently the heterochromosome, is found in the spermatids (Fig. 22), lying against the nuclear membrane, and in some cases the dimorphism can be distinguished.

Theobaldia incidens.

Theobaldia incidens is one of the most common mosquitoes in the Santa Clara Valley, California. The larvæ were found in abundance in a bucket of water which had been allowed to stand partly covered for two or three weeks. A few bread crumbs thrown into the glass jars in which the larvæ were kept under observation, enabled them to grow rapidly and transform into fine large pupæ whose germ glands yielded all desired stages, from dividing oögonia and spermatogonia to ripe spermatozoa.

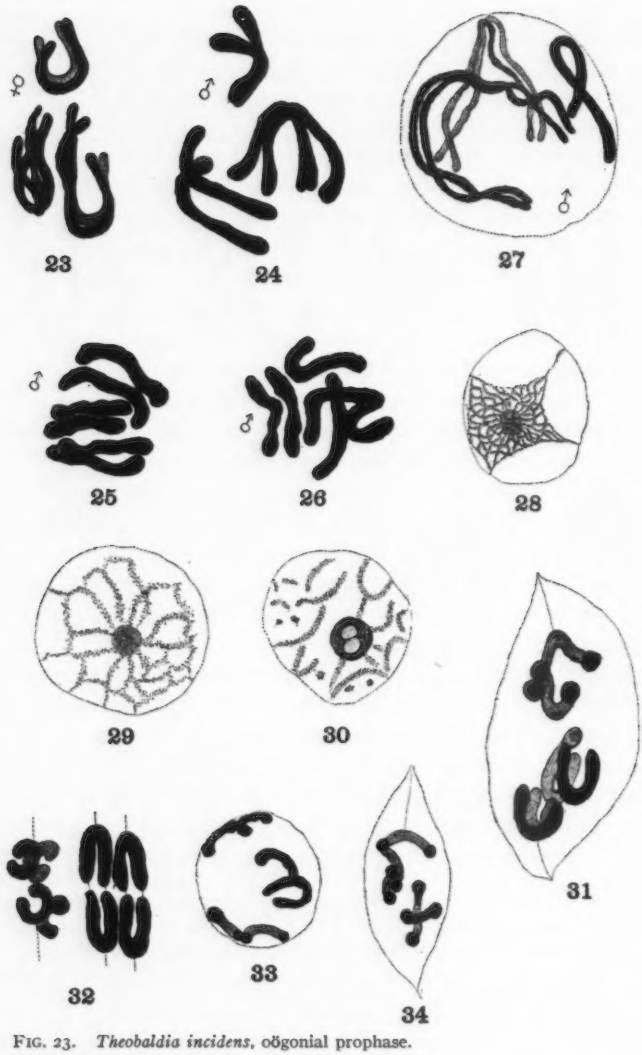


FIG. 23. *Theobaldia incidens*, oögonial prophase.
FIGS. 24–26. Chromosomes of the spermatogonia.
FIG. 27. Spermatogonial prophase.
FIG. 28. Synizesis stage.
FIGS. 29 and 30. First spermatocyte growth stages.
FIGS. 31 and 32. First spermatocyte prophase and metaphase.
FIGS. 33 and 34. Second spermatocyte prophase.

During the summer only acetocarmine preparations were used and the material resembled *Culex* so closely that only a few figures were made. Other figures were taken from sections of ovaries and testes fixed in Flemming and stained with thionin. An oögonial prophase is shown in Fig. 23, and the chromosomes from three spermatogonia in Figs. 24, 25 and 26. Fig. 27 is the nucleus of a spermatogonium in prophase. In no one of these figures is there any indication of inequality in the members of any pair of chromosomes. Neither in resting spermatogonia nor in the growth stages of the spermatocytes could anything resembling the heterochromosomes of *Anopheles* be found. In all of these stages a central plasmosome is surrounded by a spireme as in *Culex*. Figs. 28 to 30 show a contraction or synizesis stage, and two later growth stages. As in *Culex* the plasmosome stains a deeper blue in late growth stages than in the spermatogonia and synizesis stages. Fig. 31 is a characteristic prophase of the first spermatocyte mitosis. In the metaphase two of the chromosme pairs nearly always appear in the form of rings and separate as V's; the third pair is usually separated and its univalent components already divided longitudinally, when the rings are just dividing (Fig. 32). The second spermatocyte prophases (Figs. 33 and 34), metaphases and anaphases resemble closely those of *Culex*.

The testes of *Theobaldia* differed from those of *Culex* in having much larger spermatogonia and in lacking a prolonged prophase of the first spermatocyte mitosis. The first maturation division also shows characteristic differences in the behavior of the chromosome pairs in metakinesis (compare Figs. 19 and 32). Otherwise both ovaries and testes showed similar conditions in the two species, *Culex pipiens* and *Theobaldia incidens; i. e.*, three equal pairs of chromosomes, no one of which behaves in any respect like the heterochromosomes of other insects, including *Anopheles punctipennis*.

Anopheles sp.?

On March 30 and again on May 31, a few pupæ were obtained from the same pond where I obtained my material in 1909. All of the males in these collections had six chromosomes in the spermatocytes and a distinct heterochromosome in the growth

stages. The pupæ were thought to be *Anopheles* but no larvæ or adults were secured for identification. It was supposed that the few specimens obtained must have wintered over, probably in

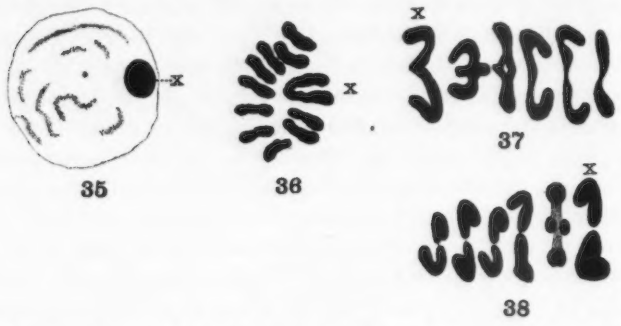


FIG. 35. *Anopheles* sp.? First spermatocyte growth stage. *X* = the heterochromosome.

FIG. 36. First spermatocyte prophase.

FIGS. 37 and 38. Chromosomes of the first spermatocyte metaphase. Mag. 2,000 in all figures.

the larval stage. The net was used in the pond many times during March, April and May, but no mosquito larvæ or pupæ secured except on the two dates given above.

Fig. 35 shows the heterochromosome in the nucleus of a growth stage, Fig. 36 a prophase of the first maturation mitosis, and Figs. 37 and 38 the six chromosomes from two first spermatocyte spindles. The largest pair is slightly unequal but not composite as in *Anopheles punctipennis*.

DISCUSSION.

As we have already seen, the various species of Culicidæ, thus far studied, present peculiarities not hitherto encountered in the study of heterochromosomes in the various groups of insects.

In *Anisoloba maritima* (Randolph, '08) and several species of Lepidoptera (Stevens, '06; Dederer, '07; Cook, '10) there is an equal pair of heterochromosomes distinguishable as such by their condensed condition in the growth stages of the first spermatocytes. In *Drosophila ampelophila* the unequal pair of heterochromosomes of the maturation mitoses is not condensed

during the growth stages (Stevens, '08). In *Metapodius terminalis* (Wilson, '09) we find either an unpaired heterochromosome or an unequal pair of idiochromosomes, and a varying number of supernumeraries within the same species. In no case before have undoubted heterochromosomes, following the usual rule as to sex distribution, been found in one or more genera or species and entirely absent in nearly related species of the same family. In *Anopheles punctipennis* the heterochromosomes can be traced from the youngest spermatogonia observed, through the two generations of spermatocytes to the spermatids and even to a stage in which the spermatids are transforming into spermatozoa. The unequal pair in the male is represented by an equal pair in the female, and as in other similar cases, it is evident that an egg fertilized by a spermatozoön containing the smaller heterochromosome must produce a male, while an egg fertilized by a sperm containing the larger heterochromosome will develop into a female.

Now in *Culex* and *Theobaldia* all of the chromosomes behave alike so far as condensation is concerned, and there is neither an unpaired heterochromosome nor an unequal pair to be detected in any stage. In other words there is no visible differentiation of one pair of chromosomes which can be associated with the determination of sex. There seems to be absolutely no reason for supposing that the mechanism by which sex is determined is not identical in these three species of mosquitoes; and the inevitable conclusion would seem to be either that the heterochromosomes of the male are not a necessary factor in sex determination, or that the sex-determining chromosomes are not necessarily differentiated as heterochromosomes.

I have kept no record of the sex of the pupæ dissected, but should say that the numbers of males and females were about equal for all of the species studied. In a large collection of *Theobaldia* larvæ which, judging by their size, might have all come from one laying, most of the males pupated a day or two sooner than the females. All of the pupæ were taken out and dissected each day. The first day nearly all were males and the last day nearly all females. An average for the four or five days, I feel sure, must have given nearly equal numbers.

Wilson's recent hypothesis ('09) that in some way two X chromosomes determine the female sex, and one X chromosome or an X and a Y chromosome determine the male sex would apply perfectly to *Anopheles*.

Egg X+sp. X=♀,
Egg X+sp. Y=♂.

But what shall we say of *Culex* and *Theobaldia* where there is no differentiation of X and Y chromosomes? In forms which have an equal pair of heterochromosomes, showing the same general characteristics as the unpaired heterochromosomes and the idiochromosomes, one naturally infers that the members of the equal pair in the male have X and Y values respectively, but shall we go further and argue that likewise in *Culex* and *Theobaldia* the mature eggs all have an X chromosome and the spermatozoa are dimorphic, one half having an X chromosome and the other half a Y chromosome, although nothing of the kind can be traced? If we make this assumption, then *Culex* and *Theobaldia* may stand at one end of a series, as examples of absence of heterochromosomes associated with sex, while forms with an unpaired heterochromosome come at the other end of the series, and all the varieties of paired heterochromosomes between, the mechanism of sex determination being the same in all, but evident to the eye only when differential heterochromosomes are present.

The case of the aphids and phylloxerans has been the strongest argument for the hypothesis that two X chromosomes give a female and one X or XY a male, since the rejection of one X chromosome from a parthenogenetic egg is followed by the development of a male, but in *Culex* and *Theobaldia* it is evident that sex determination is not dependent on the presence of X and Y chromosomes, although in *Anopheles* these chromosomes are at least closely correlated with sex-determination.

At present, the all-important questions seem to me to be: What is the meaning of the differentiation of heterochromosomes in one form and not in others closely related? What has been the history of such differentiation where we have an unpaired heterochromosome or an unequal pair of heterochromosomes?

In *Metapodius* Wilson ('09) traces the origin of an unpaired heterochromosome and also of the supernumeraries to an irregular mitosis in which both idiochromosomes go to one daughter cell. In the *Diabroticas* the supernumeraries probably owe their origin to an irregular division of the unpaired heterochromosome.

But in no case are we able to say when or how or why certain spermatogonial chromosomes became specially differentiated as heterochromosomes.

We have associated the *X* and *Y* chromosomes of the male with sex-determination, but possibly they have some other meaning and are only so correlated with the sex-determining mechanism that they have the same distribution as the sex characters. The case of the mosquitoes certainly indicates that we must study the heterochromosomes, apart from the idea of sex-determination, more intensively, in order to determine if possible why we have such chromosomes differentiated in some forms and not in others.

In such cases as *Anopheles* the sex characters may be supposed to be located in the unequal heterochromosomes or in the larger equal pair with which they are connected. This may also be true of the composite heterochromosomes of *Drosophila ampelophila* and *Hesperotettix* (McClung, '05). In the ordinary cases of an unpaired heterochromosome or an unequal pair, we may suppose the sex characters to be located in the heterochromosomes, but to be independent of the special differentiation, which may be entirely absent as in *Culex* and *Theobaldia*.

If we apply Morgan's new sex formula—

$$FmFm = ♀, \quad Fmfm = ♂,$$

to the cases under discussion, *m* may be located in any equal pair but *F* must be either in the heterochromosomes or in chromosomes correlated with them. Sex-limited inheritance in the male (Morgan, '10) would then seem to be possible only where unpaired or unequally paired heterochromosomes are present, and not in such cases as, *Anisoloba*, the Lepidoptera, *Culex* and *Theobaldia*.

It occurs to me as possible that heterochromosome differentiation may be directly related to sex-limited inheritance of certain

characters. If so we should expect to find an unpaired or an unequally paired heterochromosome in the female of *Abraxas* where there is sex-limited inheritance in the female, and also to find sex-limited inheritance of some character or characters in the female of those echinoderms in which Baltzer ('09) and Heffner ('10) have shown that an unequal pair of chromosomes is present in the female. Apparently we have reached a point where the experimental breeder and the cytologist *must* work together in order to solve the problems of sex-determination, sex-limited inheritance and heterochromosome differentiation.

BRYN MAWR COLLEGE,
December 20, 1910.

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PRELIMINARY NOTE ON HETEROCHROMOSOMES IN THE GUINEA PIG.

N. M. STEVENS.

It is the purpose of this preliminary note on the spermatogenesis of the common guinea-pig merely to state some of the most obvious facts concerning the unequally paired heterochromosomes as they appear in the first spermatocytes. In the later growth stages a characteristic heterochromosome (Fig. 1, *x*)

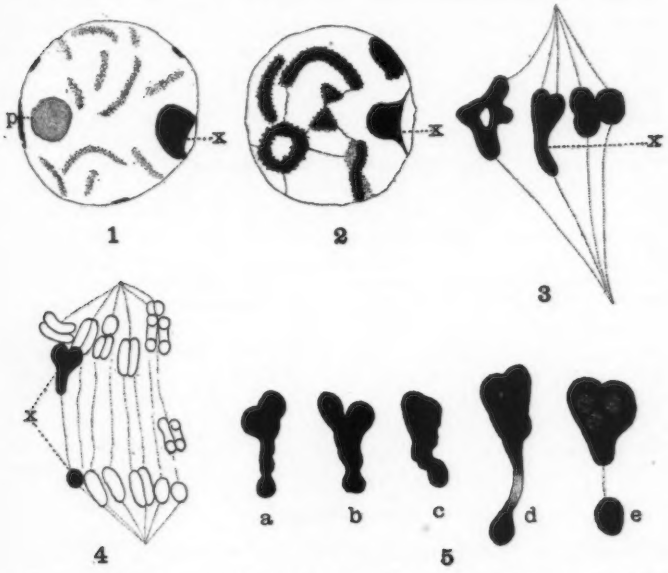

FIG. 1. First spermatocyte growth stage. *X* = the heterochromosome. Mag. 2,000.

FIG. 2. Prophase.

FIG. 3. First spermatocyte metaphase, tangential section of spindle showing the heterochromosome *X*.

FIG. 4. Anaphase.

FIG. 5. The heterochromosome; *a*, *b*, *c*, from sections of Flemming material; *c*, *d*, from acetocarmine preparations. Mag. 2,000 for all of the figures.

is easily distinguished both in acetocarmine preparations and in sections of Flemming material stained with thionin. The plasmosome (*p*) is even paler than the spireme. In Fig. 2, a prophase, the heterochromosome (*x*) is also shown. Fig. 3, a tangential section of a first spermatocyte spindle, shows the usual form of the heterochromosome in metaphase, and in Fig. 4 we have it divided into its unequal components. Fig. 5, *a*, *b*, *c*, shows slight variations in the metaphase form of the heterochromosome as seen in sections, and *d*, *e* the metakinesis of this chromosome, the large size of these two figures being due to the fact that the figures were drawn from acetocarmine preparations. Further details will be given later.

BRYN MAWR COLLEGE,
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